REMARKS/ARGUMENTS

These Remarks are responsive to the Office Action mailed November 9, 2004 ("Office Action"). Claims 1, 5-8, 10-11, 14-29, 31-45, and 50 are pending in the application. Claims 1, 6, 10-11, 14-16, 20 and 25 are amended and claim 50 is new. Claims 3-4, 9, 30 and 49 are cancelled. Support for the amended and newly claimed subject matter may be found in the specification and claims as originally filed. Specific support for the amended claims may be found at page 6, lines 13-15 and page 7, first paragraph of the specification as originally filed as well as originally filed claim 4. Applicants respectfully request reconsideration of the rejection of the pending claims for the following reasons.

Overview of the Invention

Generally, the claimed invention is directed toward determination of concentration of sperm cells and the proportion of live sperm cells (viability) of a semen sample in the same determining step. The total number of sperm cells, i.e., absolute concentration, and the proportion of live sperm cells are important factors for achieving optimal fertility. It has been found that there seems to be a cut-off value below which the fertility drops below a certain percentage thus being below the profitable fertility rates. The total number of sperm cells and the proportion of live sperm cells required for obtaining this minimum acceptable fertility is different for different species. Furthermore, the total number of sperm cells and the proportion of live sperm cells differ between males of the same species and between different ejaculates for the same male, thus leading to a cut-off value differing between males of same species and between different ejaculates for the same male. Accordingly, the claimed invention provides a method for performing a determination for each ejaculate to obtain a maximum number of insemination doses from each ejaculate.

The precision with which the total concentration of sperm cells and the proportion of live sperm cells is determined is of great importance, since a precise determination of these values provides: (a) the possibility of evaluating single ejaculates on the basis of a single determination, and (b) the possibility of using ejaculates having values closer to any predetermined cut-off value, since a more precise determination means that the safety distance to any predetermined

cut-off value may be chosen according to the precision with which the determination is performed.

The determination of the total concentration of sperm cells and the proportion of live sperm cells from a single sample increases the precision of the measurement in that the possibility of mistakes and errors during handling and pipetting are reduced. In the claimed invention, only one sample is prepared for determination whereas prior art methods use more determination routines and more measurements on samples prepared differently to obtain the total concentration and the proportion of live cells. As a result, the sources of errors when handling and pipetting the samples and when measuring the number of samples are increased and the variations on the resulting determinations are thus equally increased. Therefore, when the methods used for determination of the total sperm concentration are separate from the determination of the proportion of live sperm cells, the variation will necessarily be larger than by combining the two determinations in a single measurement. However, for some purposes it may be useful or desirable to make more determinations on the same ejaculate to exclude possible determination failures. Generally, however, a single determination should suffice.

Anticipation -- 35 U.S.C. § 102

"A claim is anticipated only if each and every element as set forth in the claim is found, either expressly or inherently described, in a single prior art reference." Manual of Patent Examining Procedure § 2131 (8th ed., rev. 2, May 2004) (quoting Verdegaal Bros. v. Union Oil Co. of California, 814 F.2d 628, 631, 2 USPQ2d 1051, 1053 (Fed. Cir. 1987)).

The Office Action rejects claims 1, 3, 5, 6, 9, 31, 45, and 49 under 35 U.S.C. § 102(b) as being clearly anticipated by Yamamoto et al., "Estimation of rat sperm with flow cytometer (FCM): simultaneous analysis of sperm number and sperm viability," Teratology, 54:38 A, 1996 ("Yamamoto"). Claim 1 requires, among other limitations, "applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells" and "determination of the absolute concentration of sperm cells and of the proportion of live sperm cells in the semen sample . . . simultaneously in the same determination step." In contrast, Yamamoto discloses a method whereby viable sperm are unstained while dead sperm are stained with propidium iodide (PI). Yamamoto uses only

one staining agent, while the claimed invention requires two staining agents. Claim 1 is thus novel over Yamamoto. Claims 3, 9 and 49 are cancelled. Claims 5, 6, 9, 31, and 45 are likewise novel over Yamamoto in that they depend from and include all of the limitations of independent claim 1. Accordingly, the rejection of pending claims 1, 5, 6, 31, and 45 as clearly anticipated by Yamamoto under 35 U.S.C. § 102(b) is improper and must be withdrawn.

The Office Action rejects claims 1, 3-10, 16-19, 28, 31, 45, and 49 under 35 U.S.C. § 102(b) as being clearly anticipated by U.S. Patent No. 4,559,309 ("Evenson"). Claim 1 requires, among other limitations, "applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells" and "determination of the absolute concentration of sperm cells and of the proportion of live sperm cells in the semen sample . . . simultaneously in the same determination step." In contrast, Evenson discloses a process whereby a sperm sample is stained with Rhodamine 123 which stains the mitochondria in the midpiece in live sperm and with ethidium bromide which stains the DNA of the dead sperm. Unlike the claimed invention, the staining agents of Evenson do not both bind to DNA. Furthermore, and as explained on page 4, lines 1-7, of the present specification, by using the R123 and ethidium bromide two different cellular compartments are targeted. A proportion of sperm in a given semen sample will lose the sperm head while the tail is still moving (detached heads). In such a sample, both the number of dead sperm and the number of viable sperm will be overestimated since the two compartments (head and tail) are separated and stained with respectively ethidium bromide and R123. Both the viability and the sperm concentration would therefore be determined inaccurate, especially for samples with 10 to 20 % detached heads. Claim 1 is thus novel over Evenson. Claims 3, 9, and 49 are cancelled. Claims 4-8, 10, 16-19, 28, 31, and 45 are likewise novel over Evenson in that they depend from and require all of the limitations of independent claim 1. Accordingly, the rejection of pending claims 1, 4-8, 10, 16-19, 28, 31, and 45 as clearly anticipated by Evenson under 35 U.S.C. § 102(b) is improper and must be withdrawn.

The Office Action rejects claims 1, 3, 5-9, 20-24, 28, 29, 31, 45, and 49 under 35 U.S.C. § 102(b) as being clearly anticipated by Takizawa et al., "Flow Cytometric Analysis for the Evaluation of the Rat Sperm Viability and Number in the Male Reproductive Toxicity Studies," Cong. Anom. 35:177-187 1995 ("Takizawa"). Claim 1 requires, among other limitations,

"applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells" and "determination of the absolute concentration of sperm cells and of the proportion of live sperm cells in the semen sample . . . simultaneously in the same determination step." In contrast, Takizawa discloses a method whereby viable sperm are unstained while dead sperm are stained with propidium iodide (PI). Takizawa uses only one staining agent, while the claimed invention requires two staining agents. Claim 1 is thus novel over Takizawa. Claims 3, 9, and 49 are cancelled. Claims 5-8, 20-24, 28, 29, 31, and 45 are likewise novel over Takizawa in that they depend from and require all of the limitations of independent claim 1. Accordingly, the rejection of pending claims 1, 5-8, 20-24, 28, 29, 31, and 45 as clearly anticipated by Takizawa under 35 U.S.C. § 102(b) is improper and must be withdrawn.

Obviousness -- 35 U.S.C. § 103(a)

"To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations." M.P.E.P. § 2143.03. "Obviousness can only be established by combining or modifying the teachings of the prior art to produce the claimed invention where there is some teaching, suggestion, or motivation to do so found either explicitly or implicitly in the references themselves or in the knowledge generally available to one of ordinary skill in the art." M.P.E.P. § 2143.01; see also In re Lee, 277 F.3d 1338, 1342-44, 61 USPQ2d 1430, 1433-34 (Fed. Cir. 2002). Finally, "[w]hen evidence of secondary considerations such as unexpected results is initially before the Office, for example in the specification, that evidence should be considered in deciding whether there is a prima facie case of obviousness." M.P.E.P. § 2144.08.

The Office Action rejects claims 1, 3-11, 14-24, 28, 29, 31, 37, 44, 45, and 49 under 35 U.S.C. § 103(a) as obvious over Takizawa in view of Live/Dead Sperm Viability Kit, L-7011, Molecular Probes Product Information 08/11/99 or Garner et al., "Viability Assessment of

Mammalian Sperm Using SYBR-14 and Propidium Iodide," Biology of Reproduction 53, 276-284 (1995) ("Garner").

Takizawa teaches a process of measuring sperm viability and counts in rats. As discussed above, the claimed invention differs from Takizawa by requiring "applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells" and "determination of the absolute concentration of sperm cells and of the proportion of live sperm cells in the semen sample . . . simultaneously in the same determination step." The Office Action relies on the Live/Dead Sperm Viability Kit or Garner to cure the foregoing deficiencies of Takizawa.

The Live/Dead Sperm Viability Kit is concerned merely with distinguishing between live and dead cells in a semen sample. Nothing in the Live/Dead Sperm Viability Kit teaches or even suggests determining the absolute concentration of sperm cells, much less doing so "in the same determination step" as determining "the proportion of live sperm cells in the semen sample" as claimed. Garner is directed to a dual staining technique for determining the proportion of living cells to dead cells in a sample. Garner fails to teach or suggest determining the "absolute concentration" of sperm cells, much less doing so "in the same determination step" as determining "the proportion of live sperm cells in the semen sample" as claimed.

The Office Action asserts that it would have been obvious to combine the test for distinguishing between live and dead cells of the Live/Dead Sperm Viability Kit or Garner with the sperm concentration measurement of Takizawa. Specifically, the Office Action at page 3 states:

> The substitution of two distinct fluorescent dyes for the PI in the method of Takizawa *et al.* would have been obvious when the primary reference was taken with Garner et al. or Live/Dead Sperm Viability Kit which both teach the superiority of their dual staining method to determine sperm viability.

The Office Action, however, fails to point to any motivation whatsoever for making the proposed combination such that both viability and counting tests are performed "in the same determination step" as claimed. The only source of information that would have motivated a person of ordinary skill in the art to practice the invention on this record comes from Applicants' own specification. Indeed, the Office Action points to nothing in the prior art suggesting that a person

of ordinary skill should determine the concentration of sperm cells and the proportion of live sperm cells in the same determination step as claimed. As discussed above, "[t]he teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, not in applicant's disclosure." M.P.E.P. § 2143 (citing In re Vaeck, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991)). Thus, the rejection of claim 1 as obvious under 35 U.S.C. § 103(a) in view of Takizawa in view of Live/Dead Sperm Viability Kit or Garner is improper and must be withdrawn. Claims 3-4, 9, and 49 are cancelled. Claims 5-8, 10-11, 14-24, 28, 29, 31, 37, 44, and 45 are likewise non-obvious over Takizawa in view of Live/Dead Sperm Viability Kit or Garner in that they depend from and require all of the limitations of independent claim 1. Accordingly, the rejection of pending claims 1, 5-8, 10-11, 14-24, 28, 29, 31, 37, 44, and 45 under 35 U.S.C. § 103(a) as obvious over Takizawa in view of Live/Dead Sperm Viability Kit or Garner is improper and must be withdrawn.

Applicants agree with Examiner Saucier's assessment that "the precision of treatment C in the declaration is impressive." See Office Action, page 7; Declaration by Preben Christen and Torben Greve dated February 20, 2004 ("Declaration"). The evidence presented in the Declaration is reasonably commensurate in scope with the subject matter of the amended claims. According to the treatment C in the Declaration and in the Examples in the specification, the semen sample is prepared and analyzed in the following way:

- raw semen is diluted (page 3, third paragraph of the Declaration);
- dye solution comprising a first fluorochrome binding to DNA and which stains all sperm cells (e.g., SYBR-14) combined with a second fluorochrome binding to DNA and which selectively stains dead and dying cells (page 3, first paragraph of the Declaration) is applied (it will be understood that the two stains can be exchanged with any other combination of stains capable of the same selective staining, e.g., SYTO-dyes in combination with ethidium bromide or ethidium homodimer);
- the stained sample is subjected to one pass through a detector, e.g. a flow cytometer, as described on page 3, second paragraph of the Declaration, whereby both the concentration and the % viability is determined.

The claims now require "applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells." The use of two fluorochromes capable of staining the DNA of all sperm cells and the DNA of dead and dying sperm cells, respectively, makes it possible to determine in the same determination step the absolute concentration of sperm cells and the proportion of live sperm cells in a single sample, which results in improved precision as both fluorochromes stain the same compartment of the sperm cell. This implies that even though the semen sample contains detached heads, the determination will be precise because only heads of the sperm will be counted. In the prior art methods that do not distinguish between sperm heads and other material, both the viability and the sperm concentration is inaccurately determined, especially for samples with 10 to 20% detached heads. Being able to determine these two parameters in the same analyzing step further increases the precision of the measurement in that the possibilities of mistakes and errors during handling and pipetting are reduced. The Declaration shows the enhanced precision of the claimed invention which is compelling evidence that the claimed invention is non-obvious over the prior art of record. Furthermore, the enhanced precision obtained by employment of the invention is completely unrecognized in the prior art as none of the cited references teach or suggest determination of total concentration and proportion of live sperm cells in the same determination step or any advantages of this procedure, i.e. the more precise result obtained and the usefulness of this more precise result. Thus, the rejection of claim 1 as obvious under 35 U.S.C. § 103(a) in view of Takizawa in view of Live/Dead Sperm Viability Kit or Garner is improper and must be withdrawn. Claims 3, 9, and 49 are cancelled. Claims 5-8, 10-11, 14-24, 28, 29, 31, 37, 44, and 45 are likewise non-obvious over Takizawa in view of Live/Dead Sperm Viability Kit or Garner in that they depend from and require all of the limitations of independent claim 1. Accordingly, the rejection of pending claims 1, 5-8, 10-11, 14-24, 28, 29, 31, 37, 44, and 45 under 35 U.S.C. § 103(a) as obvious over Takizawa in view of Live/Dead Sperm Viability Kit or Garner is improper and must be withdrawn.

The Office Action rejects claims 25-27 under 35 U.S.C. § 103(a) as obvious over Takizawa and Live/Dead Sperm Viability Kit or Garner as applied to claims 1, 3-11, 14-24, 28, 29, 31, 37, 44, 45, and 49 above and in combination with EP 0 586 183. Claims 25-27 depend ultimately from and incorporate the limitations of claim 1, which is nonobvious in view of

Takizawa and Live/Dead Sperm Viability Kit or Garner as set forth above. EP 0 586 183 fails to cure the deficiencies of Takizawa, Live/Dead Sperm Viability Kit, and Garner discussed above with respect to claim 1. Specifically, EP 0 586 183 fails to teach or suggest "applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells." Furthermore, the methods described in EP 585 183 for measuring the concentration in a sample were developed for measuring blood samples. As a person skilled in the art would know that live sperm cells are more sensitive than blood cells, and would not consult that document for finding a method for the precise determination of the absolute concentration and % viability in a semen sample. Accordingly, the rejection of claims 25-27 under 35 U.S.C. § 103(a) is improper and must be withdrawn.

The Office Action rejects claim 30 under 35 U.S.C. § 103(a) as obvious over Takizawa and Live/Dead Sperm Viability Kit or Garner as applied to claims 1, 3-11, 14-24, 28, 29, 31, 37, 44, 45, and 49 above and further in view of Clay. Claim 30 is cancelled thereby rendering this ground of rejection moot.

The Office Action rejects claims 32-34, 39, 41, and 42 under 35 U.S.C. § 103(a) as obvious over Takizawa and Live/Dead Sperm Viability Kit or Garner as applied to claims 1, 3-11, 14-24, 28, 29, 31, 37, 44, 45, and 49 above and further in view of Sexton or Januskauskas or Belorkar or Bostofte. Claims 32-34, 39, 41, and 42 depend ultimately from and incorporate the limitations of claim 1, which is nonobvious in view of Takizawa and Live/Dead Sperm Viability Kit or Garner as set forth above. Sexton, Januskauskas, Belorkar, and Bostofteto fail to cure the deficiencies of Takizawa, Live/Dead Sperm Viability Kit, and Garner discussed above with respect to claim 1. Specifically, neither Sexton nor Januskauskas nor Belorkar nor Bostofte teach or suggest "applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells" and "determination of the absolute concentration of sperm cells and of the proportion of live sperm cells in the semen sample . . . simultaneously in the same determination step." Indeed, Sexton, Januskauskas, Belorkar, and Bostofte are representative of the prior art wherein the viability and the concentration of sperm cells are determined in two distinct steps. In these methods, the viability is first determined and subsequently is the concentration determined after all sperm cells have been killed, regardless of the employed procedures for determining viability and

concentration. As such, Sexton, Januskauskas, Belorkar, and Bostofte fail to cure the deficiencies noted with respect to Takizawa and Live/Dead Sperm Viability Kit or Garner above. Accordingly, the rejection of claims 32-34, 39, 41, and 42 under 35 U.S.C. § 103(a) is improper and must be withdrawn.

The Office Action rejects claims 35 and 40 under 35 U.S.C. § 103(a) as obvious over Takizawa and Live/Dead Sperm Viability Kit or Garner as applied to claims 1, 3-11, 14-24, 28, 29, 31, 37, 44, 45, and 49 above and further in view of Juonala and/or Viudes-De-Castro. Claims 35 and 40 depend ultimately from and incorporate the limitations of claim 1, which is nonobvious in view of Takizawa and Live/Dead Sperm Viability Kit or Garner as set forth above. Although Juonala and Viudes-De-Castro have found a correlation between viability and litter size and concentration and litter size, respectively, the present invention provides a more precise result due to the determination of total concentration and the proportion of live sperm cells in the same determination step in each ejaculate and thus allows for rejection/acceptance of single ejaculates. Thus, Juonala and Viudes-De-Castro fail to cure the deficiencies of Takizawa, Live/Dead Sperm Viability Kit, and Garner discussed above with respect to claim 1. Specifically, neither Juonala nor Viudes-De-Castro teach or suggest "applying a first fluorochrome which binds to DNA and stains all sperm cells and a second fluorochrome which binds to DNA and selectively stains dead and dying cells" and "determination of the absolute concentration of sperm cells and of the proportion of live sperm cells in the semen sample . . . simultaneously in the same determination step." Accordingly, the rejection of claims 35 and 40 under 35 U.S.C. § 103(a) is improper and must be withdrawn.

Applicants submit that this response addresses all of the issues raised in the Office Action and places the pending claims in condition for allowance. Should any issues remain to be discussed in this application, the undersigned may be reached by telephone. In the event any variance exists between the amount authorized to be charged to the Deposit Account and the Patent Office charges for reconsideration of this application, please charge or credit any difference to the undersigned's Deposit Account No. 50-0206.

Respectfully submitted,
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